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# Liposome derived membrane adsorber for purification of nucleic acids

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DNA vaccines became a new generation of biotechnology products in the marketplace for the treatment and prevention of genetic disorders and acquired diseases. These therapies are based on the introduction of specific nucleic acids in the cells to restore, cancel, enhance or introduce a biochemical function [1]. Nonviral vectors such as the pharmaceutical grade plasmid DNA (pDNA) are a safer alternative to deliver the necessary genes for these therapies due to their lower toxicity and larger gene capacity [2].

For a DNA vaccine to be successfully developed and with the increasing demand of highly purified pDNA, optimal chromatographic techniques are required.

Membrane chromatography enables good separation efficiency and increased productivity due to negligible diffusive mass transfer to the surface [3]. The membrane adsorbers can be tailored for hydrophobic interaction chromatography (HIC) to foster pDNA purification by promoting hydrophobic interactions between pDNA (or its contaminants) and surface ligands on the separation matrix. With this application purpose, an alkyl-membrane for HIC was developed [4].

A Sartobind® Sartorious aldehyde-activated membrane was derivatized with lipidic ligands to explore new membrane-HIC adsorbers for final pDNA purification step from *Escherichia coli* lysate contaminants, namely RNA. Unillamelar liposomes of 1,2-dilauroyl-*sn*-glycero-3-phosphate (DLPA) were used for membrane derivatization based on chloroformate activation preceded by membrane preparation steps. The efficiency of lipidic bonding to the precursor membranes was followed by phosphorous analysis.

The density of DLPA ligands in the derivatized-membrane surface (0.41±0.1μeq $cm^{-2}$), analyzed through phosphorous content, is higher but eventually in the same order of magnitude of the active sites density in precursor membrane (<0.1μeq $cm^{-2}$).

Afterwards, chromatographic runs over identical DLPA membrane adsorbers were performed using the model plasmid pVAX1-LacZ (6050 bp) from clarified *E. coli* cell lysates. Plasmid DNA was separated from remaining impurities, specifically RNA (Figure 1), demonstrating the feasibility of liposome-membrane HIC for the pDNA downstream purification process. Only fractions 11-13 show strong RNA content.



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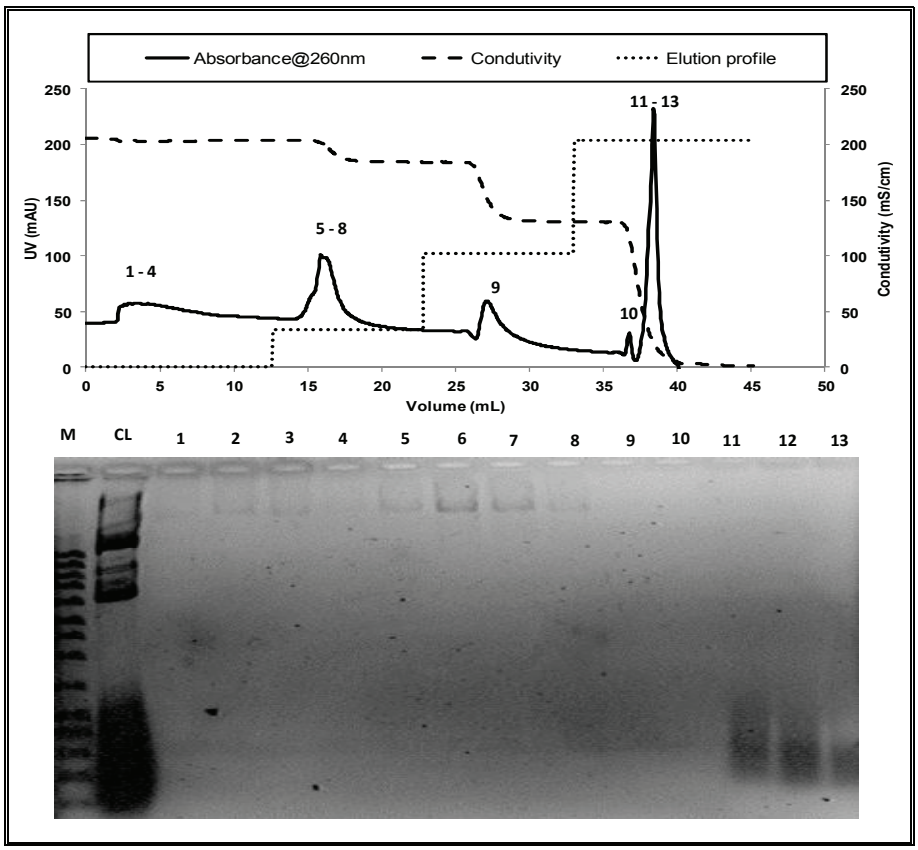


Figure 1- HIC performance of DLPA-membrane adsorber (top) and agarose gel electrophoresis (AGE) analysis of resultant chromatographic fractions (bottom). HIC specifications – Two 25mm membrane discs, 4 elution steps at 1mL/min, 100µL lysate feed solution. AGE specifications – M stands for NZYDNA Ladder IIII; CL stands for clarified lysate; fractions in each numbered lane correspond to those with same numbers in above chromatogram; delayed bands are due to high ammonium phosphate salt concentration.

To identify and characterize nucleic acids interactions with the derivatized membrane, a biophysical research by FRAP (fluorescence recovery after photobleaching) is ongoing. The spatio-temporal observation of fluorescent markers selected for both nucleic acids, in the mobile phase, and lipidic moieties, in the membrane adsorber, is under study, expecting to disclose the affinity of a liposome-functionalized membrane towards adsorption of nucleic acids.

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